11 Genetics of Host Range in Lepidoptera

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INTRODUCTION

The genetic basis of complex, ecologically relevant traits is not well known for any organism, despite enormous interest in understanding how such traits evolve. The question is particularly compelling where closely related species have diverged radically in their adaptation to the environment. Differences in host plant use among moths and butterflies often provide such cases: Although close relatives tend to use similar hosts, there are many examples of congeneric species that differ widely in host range. In several systems, work is under way to identify the genetic changes that underlie shifts in host use. While such changes may or may not contribute to the well-documented speciosity of phytophagous insects, understanding the genetic architecture of host range is fundamental to understanding the evolution of Lepidoptera. Improved understanding of the genetics of host range is crucial for applied reasons as well: Both the safe practice of biological control and the breeding of plants with persistent resistance to pests demand greater understanding of the genetics of host range. Understanding the evolution of host range in Lepidoptera will require knowledge of its genetic architecture, that is, which genes are involved, how these genes interact, and how much change in each gene is needed for a change in host range.

Host range in phytophagous insects involves not one, but many, traits. To use a plant, an insect must find and lay eggs on it and feed and develop to adulthood on it. Thus host range is multifactorial, and the competing demands of each phase of host use must be integrated. (Although the term "host range" is used in several ways in the literature, we mean the list of host plant species on which an herbivore species will oviposit and on which its larvae have some chance of completing development.) Host range is dynamic because use of a given host depends on both external factors

(e.g., local host availability, competition) and internal factors (e.g., female egg load, age, previous experience). As a result, a clear understanding of the genetic differences responsible for differences in host range is difficult to obtain because not only is host range a moving target but many genes may be involved in the numerous processes that determine host range.

The plant species on which larvae may feed are restricted by where their mothers lay eggs. Most neonate larvae can travel only a few meters in search of food before they starve, although ballooning neonates can travel greater distances. Even late instars have an ambit measured in meters to tens of meters, compared with the hundreds to tens of thousands of meters that lepidopteran adults can travel, either under their own power or carried by the wind. Thus adult females have a much greater opportunity than their progeny to choose suitable host plant species. Whether larvae themselves will have the opportunity to become adults and search for host plants for their progeny depends on larval feeding and performance on the host plant where they find themselves. As a result, host range involves genes underlying adult chemoreception and interneuronal processing, which lead to oviposition on one hand; and larval chemoreception, digestion, and nutritional metabolism, which determine larval feeding, growth, and survival on the other.

Large numbers of chromosomes and sex-limited recombination make Lepidoptera attractive models for investigations into the genetic basis of host range, as well as other complex traits. In Lepidoptera, within-chromosome recombination is restricted to males, so maternal-origin chromosomes are inherited intact (Suomalainen 1969 and references therein; Marec 1996; see Chapter 3 for details on absence of recombination in females). Genetic linkage mapping is simplified because maternal-origin "linkage groups" are actually chromosomes, and any putative recombination can be attributed to scoring error (in systems where recombination occurs, disentangling scoring error from true recombination can be a major challenge). The majority of Lepidoptera have between 28 and 32 small chromosomes of relatively uniform size (Suomalainen 1969; Robinson 1971), although chromosome numbers across Lepidoptera range from n = 5 to n = 223 (White 1973; De Prins and Saitoh 2003; see Chapter 3 for characteristics of lepidopteran chromosomes). Whereas the presence of numerous small chromosomes makes them difficult to distinguish cytologically, it also means that each chromosome comprises a relatively small fraction of the genome. If the distribution of chromosome sizes and the total number of genes in most Lepidoptera resemble estimates for the silkworm *Bombyx mori* (Xia et al. 2004; Yoshido et al. 2005), each chromosome will contain 2–5 percent of the genome or about 300 to 1,000 genes. This means that resolution to chromosome in Lepidoptera is at least as precise as in many systems having within-chromosome recombination. Finer-scale resolution can be achieved using a biphasic approach whereby one maps first to chromosome with female-informative markers and then within chromosome with male-informative markers (Heckel et al. 1999). This allows one to concentrate on chromosome(s) carrying genes of interest, a major advantage for fine-scale mapping and map-based (positional) cloning.

Over five thousand papers and books had been published on plant-insect interactions by 2002 (Scriber 2002), and the numbers continue to increase rapidly. Current knowledge on the evolutionary biology of herbivore-plant interactions has been recently and thoroughly reviewed, including phylogeny, biochemistry, behavior, and evolution (Tilmon 2008). Despite the volume of interesting research and the advantages of lepidopteran genetics discussed above, we do not know the detailed genetic architecture of host range for any species of moth or butterfly. In this chapter, we review what is known about the genetics of host range in Lepidoptera, discuss the biology of host range and its implications for genetic architecture, and suggest promising lines of research. Although we cover most thoroughly the system on which we work and thus know best—the generalist *Heliothis virescens* and the closely related specialist *Heliothis subflexa* (Noctuidae)—we treat several other systems in-depth as well. Many themes and questions that permeate the literature will become apparent in this review: adult oviposition preference versus larval performance; trade-offs in performance among host species; the pace of host range evolution; many versus few genes; genes on autosomes versus sex chromosomes; differences in the basis of interspecific versus intraspecific

variation; expansion or contraction in host range versus shifts in host range; directionality of evolution from generalist to specialist or vice versa; and the role of host shifts in speciation.

THE GENETICS OF HOST SPECIFICITY

In this section, we summarize by genus the current evidence concerning the genetics of host range. There are various types of evidence: (1) phylogenetic patterns; (2) population and strain comparisons, especially in common-garden experiments; (3) responses to artificial and natural selection; (4) crosses between host races and species; (5) resemblance of relatives (parent-offspring regression, full-sib families, half-sib families); (6) marker-based mapping of quantitative trait loci; (7) differences in sequence and expression of proteins involved in chemoreception, detoxification, and assimilation of plant chemicals. Two additional types of evidence will soon become available: map-based (positional) cloning of genes, and silencing of candidate genes.

Heliothis

The *Heliothis virescens* complex comprises at least thirteen closely related species in North and South America that vary in host specificity and geographic range (Mitter, Poole, and Matthews 1993). Among the members of this complex, two are of particular interest: *H. virescens* and *H. subflexa*. *Heliothis virescens* is a major agricultural pest and has been the subject of much research (over thirteen hundred papers in refereed journals alone). *Heliothis subflexa* is not a pest but is closely related to *H. virescens*, with which it has 99 percent sequence similarity in the genes for which comparisons have been made (Cho et al. 1995; Fang et al. 1997). Their geographical ranges overlap broadly (Mitter, Poole, and Matthews 1993), and the two species are morphologically so similar that *H. subflexa* was only conclusively identified as a separate species in 1941 (McElvare 1941). In the laboratory, *H. virescens* and *H. subflexa* can be hybridized, producing fertile F_1 females and sterile F_1 males (male fertility is restored after several backcross generations; Karpenko and Proshold 1977). These two species are thought to have evolved quite recently from a shared, generalist ancestor (Mitter, Poole, and Matthews 1993; Poole, Mitter, and Huettel 1993; Fang et al. 1997). Despite the similarity between them, they differ greatly in host range. *Heliothis virescens* has a very broad host range, feeding on at least 37 species in 14 plant families, including *Nicotiana tabacum* (tobacco), *Gossypium hirsutum* (cotton), *Glycine max* (soybean) and other crops (Sheck and Gould 1993), whereas *H. subflexa* is narrowly specialized on the genus *Physalis* (e.g., ground cherry *P. pruinosa*; Laster, Pair, and Martin 1982). Interestingly, *H. virescens* is not known to feed on *Physalis* species in the field. Thus, the *H. virescens/H. subflexa* pair is an excellent model for studying the evolution of genetic differences responsible for divergence in host range, because genetic differentiation is likely to be concentrated in loci involved in host use (Sheck and Gould 1993) and mate recognition (Groot et al. 2004).

Several studies have examined the genetic basis of host range in *H. virescens* and *H. subflexa*. Sheck and Gould (1993) analyzed larval performance on four plant species by exposing *H. virescens*, *H. subflexa*, their F_1 hybrids, and a backcross to *H. subflexa* to cotton, soybean, tobacco (hosts of *H. virescens*), and *Physalis pubescens* (a host of *H. subflexa*). Each species survived and gained weight well on its own host(s) and poorly on nonhosts. Hybrid F_1 larvae survived well on all host plants, but had intermediate weight gain on all four plant species. In the backcross to *H. subflexa*, larval survival was lower on cotton, soybean, and tobacco than on *P. pubescens*, and larval weight gain was lower on cotton and tobacco than on soybean and *P. pubescens*. Analysis of the results from the four types of cross (within each species, F_1, and backcross), indicated that genes from *H. virescens* were partially dominant for larval survival and weight gain on cotton and tobacco, but additive for both traits on soybean. Genes from *H. subflexa* were overdominant for survival and dominant for weight gain on *P. pubescens*, so that backcross larvae survived better and gained weight as well as *H. subflexa*. However, epistatic or gene-environment interactions also

appeared to be involved because additive and dominance effects alone did not explain the results. In a subsequent experiment, repeated backcrosses to *H. subflexa* with selection for larval performance on soybean were used to examine the genetic architecture for use of several plant species (Sheck and Gould 1996). After several generations of selection, larval preference and performance were tested on cotton, soybean, tobacco, and *P. pubescens*. Although performance on soybean had improved, no correlated changes occurred in performance on cotton, tobacco, or *P. pubescens*, indicating performance on these plants had an independent genetic basis. Interestingly, larval preference for soybean, though not selected on, had also increased, implying a common genetic basis for larval preference for and performance on soybean. Larval performance on *P. pubescens* did not differ from that of *H. subflexa*, showing that introgession of genes for using soybean into the *H. subflexa* background did not involve tradeoffs in ability to use *P. pubescens*.

Sheck and Gould (1995) also examined oviposition behavior of *H. virescens*, *H. subflexa*, and their reciprocal F_1 hybrids. Adult females were exposed to cotton, soybean, tobacco, and *Physalis angulata* (a favored host of *H. subflexa*) in laboratory assays. *Heliothis virescens* females oviposited mostly on tobacco and rarely on the other plant species; *H. subflexa* females oviposited mostly on *P. angulata*, but also oviposited occasionally on nonhosts; F_1 females from crosses in both directions oviposited preferentially on tobacco, indicating dominance of genes from *H. virescens*. Inheritance appeared to be autosomal with no indication of sex-linkage for genes affecting oviposition preference, larval performance, or larval preference (Sheck and Gould 1993, 1995).

In recent experiments with interspecific hybrids (unpublished collaborations between the authors and F. Gould), we have further explored the genetic basis of host range in *H. virescens* and *H. subflexa*. In laboratory experiments, we introgressed genes from each species into the background of the other species by backcrossing and assaying their backcross progeny on either cotton or *P. angulata*. Using amplified fragment length polymorphism (AFLP) markers and polymorphisms in published gene sequences, we made linkage maps covering the 31 chromosomes of *H. virescens* and *H. subflexa* (for chromosome numbers, see Chen and Graves 1970; Sheck et al. 2006) and used quantitative trait locus (QTL) analysis to determine the genetic architecture of variation in larval performance. In the experiments on cotton, we did five generations of backcrosses. For generations one to four, hybrid females were mated with *H. subflexa* males; these crosses generated female-informative markers that allowed us to identify introgressed chromosomes contributing to phenotypic variation. In generation five, hybrid males were backcrossed to *H. subflexa* females, giving us male-informative markers for within-chromosome mapping. In a preliminary analysis of first-generation backcross larvae, stepwise regression of larval feeding versus the presence/absence of *H. virescens*–origin chromosomes identified six chromosomes that together explained 39 percent of the variation in larval feeding on cotton (unpublished data). Four *H. virescens* chromosomes increased the amount of cotton consumed, and larvae with all four chromosomes had phenotypes indistinguishable from *H. virescens*. These chromosomes had additive effects with no interaction among chromosomes. Two of the introgressed *H. virescens* chromosomes had an unexpected effect: Their presence reduced, rather than increased, the amount of cotton eaten by backcross larvae. Perhaps these chromosomes carry genes for feeding on host plants other than cotton that interact epistatically with those for feeding on cotton. Because backcross larvae were either homozygous for *H. subflexa* alleles or heterozygous for *H. subflexa* and *H. virescens* alleles, the introgressed genes from *H. virescens* were at least additive and perhaps dominant. One of the sex chromosomes was among those that increased feeding on cotton, although its impact was no greater than that of autosomes. In backcrosses (BC) of hybrid females (W_vZ_s) to *H. subflexa* males (Z_sZ_s), all female progeny had their Z chromosome from *H. subflexa* and their W chromosome from *H. virescens*, but all male progeny had both sex chromosomes from *H. subflexa*. This means either that there were genes on the W_v chromosome that increased feeding on cotton, which seems unlikely given the paucity of expressed genes on the W chromosome (see Chapters 3 and 4 for candidate W-linked genes and molecular composition of the W chromosome), or there was an overall difference in feeding between the sexes. Although we have not yet mapped QTL in BC_5

larvae, the frequency of BC_5 larvae with *H. virescens*–like phenotypes indicates that a few genes explain much of the variance in feeding on cotton. Recent theory and evidence suggest that finding few QTL that explain most of the variation in quantitative traits is not surprising (Orr 2001, 2005; Remington, Ungerer, and Purugganan 2001).

To investigate the genetics of larval performance on *P. angulata*, we introgressed *H. subflexa* genes into the *H. virescens* background by backcrossing hybrids to *H. virescens*. When fed on the fruits of *P. angulata*, the assimilation efficiency (larval weight gain per gram of fruit consumed) of *H. subflexa* is thirty times greater than that of *H. virescens*, although *H. virescens* larvae feed readily on *P. angulata*. The phenotypes of backcross larvae ranged from *H. subflexa*–like to *H. virescens*–like. Five introgressed chromosomes affected the performance of backcross larvae on *P. angulata*, together explaining 45 percent of the variation in assimilation efficiency. Similar to the cotton results, three chromosomes increased assimilation efficiency, while two decreased assimilation efficiency. The presence of the three chromosomes that increased assimilation efficiency gave phenotypes equal to those of *H. subflexa* (unpublished data).

Much effort has been directed toward understanding how *H. virescens* detects and selects host plants and mates. Twenty-one genes coding for olfactory receptor proteins, each from a different group of olfactory neurons, have been sequenced in *H. virescens* (Krieger et al. 2002, 2004). Antennal lobe structure and patterns of innervation suggest that there at least thirty to sixty types of olfactory neurons and thus olfactory receptor proteins (Mustaparta 2002; Rostelien et al. 2005). Furthermore, sixteen types of olfactory neurons have been identified based on their electrophysiological response to plant odors, and all are finely tuned to specific plant odors (Rostelien et al. 2005). These receptor genes may provide candidates for explaining differences in host specificity between these two species.

Helicoverpa

Like *Heliothis*, the genus *Helicoverpa* (see Chapter 12 for further information on this genus) includes species with broad host ranges such as *H. armigera*, recorded from over 150 host plant species in many families (Zalucki et al. 1994), and *H. zea*, recorded from at least 34 species of plants in 11 families (Sudbrink and Grant 1995), as well as species with narrow host ranges like *H. assulta*, recorded from only certain species in the Solanaceae (Fitt 1989). Laboratory experiments have been used to examine population variation and heritability of oviposition preference and larval performance in these species. Populations of *H. armigera* from various regions of Australia did not differ in ranking of plant species (maize, sorghum, tobacco, cotton, cowpea, lucerne) for oviposition, but females within populations did show heritable variation (parent-offspring regression) in ranking of these plants (Jallow and Zalucki 1996). Besides showing genetic variation in oviposition among plant species, female *H. armigera* also appear to learn: Females oviposit preferentially on plant species previously experienced (Cunningham et al. 1998). In another laboratory study on Australian *H. armigera*, a full-sib parent-offspring regression showed high heritability (60 percent) for oviposition on *Sonchus oleraceus* (Asteraceae), a preferred host plant from the indigenous geographical range of *H. armigera* (Gu and Walter 1999), versus *Gossypium hirsutum* (Malvaceae), a less-preferred host plant (Gu, Cao, and Walter 2001). Although *H. armigera* larvae survived better and gained more weight on *S. oleraceus* than on *G. hirsutum*, larval performance was not genetically correlated with oviposition preference (Gu, Cao, and Walter 2001). In a full-sib/half-sib experiment on Australian *H. armigera*, larvae gained more weight (73 percent for neonates and 23 percent for third instars) but did not differ in survival on resistant versus susceptible *Cicer arietinum* (chickpea; Cotter and Edwards 2006). Heritability was high for larval weight gain on both resistant and susceptible varieties, but heritability was zero for oviposition, and females did not distinguish between varieties in oviposition.

In laboratory experiments, populations of *Helicoverpa zea* from different regions of North America (where the moth is indigenous) differed in ranking of plant species and varieties (hairy

vs. glabrous soybean and cotton) for oviposition, and within the one population tested oviposition preference was heritable (although with large variance; Ward et al. 1993).

In the most interesting experiment concerning the genetics of host range in *Helicoverpa*, interspecific crosses (F_1, F_2, and backcrosses) of the generalist *H. armigera* and specialist *H. assulta* indicated that at least one major autosomal gene was involved in larval feeding on cotton and that *H. armigera* alleles were partially dominant to *H. assulta* alleles (Tang et al. 2006).

Papilio

The genus *Papilio* broadly construed comprises about 205 species (which may, in fact, represent as many as six genera) whose ancestors appear to have fed on species in the Rutaceae; 80 percent of species still feed on plants in this family (Zakharov, Caterino, and Sperling 2004). However, several clades have diverged from Rutaceae use, including the *glaucus* complex (*Papilio* [*Pterourus*] *glaucus*, *P. canadensis*, and related species), which attack species in at least eight plant families (Bossart and Scriber 1995a), and the *machaon* complex (*Papilio machaon, P. zelicaon*, *P. oregonius*, and related species), which attack species in Apiaceae (Umbelliferae) and Asteraceae (Sperling and Harrison 1994).

Papilio zelicaon is reported from over sixty species of Apiaceae and Rutaceae (Wehling and Thompson 1997), but *P. oregonius* is reported only from a single species (*Artemisia dracunculus*) of Asteraceae (Thompson 1988). In laboratory experiments with *P. zelicaon*, *P. oregonius*, and their reciprocal interspecific F_1 hybrids, females of each species showed strong oviposition preference for the appropriate field hosts; but their hybrids showed preferences similar to that of their paternal source, indicating a major locus or loci on the Z sex chromosome, although genes on autosomes modified preferences (Thompson 1988). Larval survival of each species was high on the appropriate plant, but survival of hybrid larvae was intermediate on both host plants, indicating autosomal inheritance of genes with additive effects (Thompson, Wehling, and Podolsky 1990). On the other hand, hybrid pupal mass and to a lesser extent development time were closer to the maternal source, indicating maternal effects, but not sex linkage (Thompson, Wehling, and Podolsky 1990).

In laboratory analyses of oviposition preference hierarchies among five *machaon*-complex species for five species of Apiaceae and Asteraceae, the butterflies showed a range of preference hierarchies from narrow to broad (Thompson 1998). One pair of sister species (*P. machaon*/*P. oregonius*) differed strongly in ranking of plant species, with *P. machaon* laying eggs on most species and *P. oregonius* laying only on a plant barely used by *P. machaon*, while another pair of sister species (*P. polyxenes*/*P. zelicaon*) closely resembled one another in ranking of plant species (Thompson 1998). Populations within *P. machaon*, and to a lesser extent within *P. polyxenes* and *P. zelicaon*, differed somewhat in preference hierarchies, and these differences may provide the raw material for host range shifts (Thompson 1998). For example, a few females in some populations of *P. machaon* laid a few eggs on *A. dracunculus*, the only known host of *P. oregonius*. The shift by *P. oregonius* to ovipositing on this plant may have been easy because oviposition preference in *P. oregonius* appears to be sex-linked and may involve few loci (Thompson 1988, 1998). However, this does not explain why the shift to *A. dracunculus* by *P. oregonius* led to dropping other plants from its host range. Although there was a shift toward local plants in populations of *P. zelicaon*, butterflies did not strongly prefer local plants, despite genetic variation in preference within these populations, as determined by differences among full-sib families (Thompson 1993; Wehling and Thompson 1997). This lack of strong preference for local hosts may result from coadapted gene complexes involved in preference for certain plants, from gene flow among populations preventing a response to selection, or from a lack of strong selection for adaptation to local hosts (Thompson 1993; Wehling and Thompson 1997).

Bossart (1998, 2003) and Bossart and Scriber (1995a,b, 1999) conducted a series of laboratory experiments on differences in oviposition preference and larval performance on three tree species (*Liriodendron tulipifera*, *Magnolia virginiana*, and *Prunus serotina*) among geographical

populations of *P. glaucus* with different field exposures to these plants. Females from Florida, where *M. virginiana* is common and *L. tulipifera* rare, oviposited more on *M. virginiana* than females from regions where *L. tulipifera* was common and *M. virginiana* rare (Georgia) or absent (Ohio; Bossart and Scriber 1995a). However, like *P. zelicaon* in California (Thompson 1993), Florida *P. glaucus* did not strongly prefer to oviposit on the local host. The Ohio population showed very high heritability (0.81) in oviposition preference between *L. tulipifera* and *M. virginiana*, with some families ovipositing on both trees and some on *L. tulipifera* only (Bossart and Scriber 1999). Larvae from Florida and Georgia performed better on *M. virginiana* (as measured by development time and pupal mass) than larvae from Ohio, indicating adaptation to a locally available host, although larvae from all three regions still did best on *L. tulipifera* (Bossart and Scriber 1995a; Bossart 2003). These geographical populations did not differ in allozyme frequencies, which suggests that gene flow between them is counteracted by local selection to maintain differences in oviposition preference and larval performance (Bossart and Scriber 1995a). Comparison of larval performance among full-sib families showed no heritability for larval performance on *M. virginiana* for the Florida population, but significant heritability for performance on this host for the other two populations, as well as for performance on *P. serotina* for all three populations (Bossart 1998). Performance on the three hosts was either genetically uncorrelated or positively correlated, indicating no trade-offs in host plant suitability (Bossart 1998). In the locally polyphagous Ohio population, larvae from mothers that oviposited preferentially on *L. tulipifera* did better than larvae from mothers that oviposited preferentially on *M. virginiana*, regardless of the host plant on which they were reared, revealing a negative correlation between preference versus performance on *M. virginiana* (Bossart 2003). This may not be surprising given that the Ohio population is not exposed to *M. virginiana*, so selection for a preference-performance correlation is lacking. In the locally monophagous Florida population, oviposition preference showed no correlation with three of the four measures of larval performance and a negative correlation with the fourth measure (Bossart 2003), which suggests that selection may have shifted both oviposition preference and larval performance (Bossart and Scriber 1995a), but not enough to have resulted in a positive relationship. Although the preference-performance relationship appears to have a genetic basis, it is not the relationship expected from optimal oviposition theory, perhaps because of constraints arising from coadaptation, pleiotropy, or epistasis among genes controlling both preference and performance (Bossart 2003).

Differences in regulation and activity of cytochrome P450 monooxygenases have been implicated in differences in host use among Lepidoptera in general and papilionids in particular (for review, see Berenbaum and Feeny 2008). *Papilio polyxenes* specializes on species of Apiaceae and Rutaceae with high levels of specific furanocumarins (xanthotoxin and angelicin) and has high activity of P450s specific for these allomones that are not very effective at metabolizing others; *P. glaucus* and *P. canadensis* have broader host ranges and have P450s that metabolize a variety of allomones with less efficiency but are highly inducible (Li, Schuler, and Berenbaum 2007). Differences in P450 regulation and activity between *P. glaucus* and *P. canadensis* may play a role in the differences in their host ranges (Li, Schuler, and Berenbaum 2003).

Euphydryas

The genus *Euphydryas* (Nymphalidae) in the broad sense comprises fourteen species (Zimmermann, Wahlberg, and Descimon 2000). Their larvae feed on plant species in five families that produce iridoids, and Neartic *Euphydryas* specialize on plants of the families Scrophulariaceae and Plantaginaceae that have iridoid glycosides that the butterflies sequester (Zimmermann, Wahlberg, and Descimon 2000, and references therein). These butterflies disperse little and show interpopulation variation in host plant use. These attributes, as well as oviposition behavior that can be manipulated and measured in the field, led to a series of studies on the genetics and evolution of host use, particularly in *E. editha*. Several rapid shifts in plant species by various populations of *E. editha* have been documented (for review, see Singer et al. 2008). One population shifted from

most females ovipositing on a native plant, *Collinsia parviflora* (Scrophulariaceae), to most females ovipositing on an introduced plant, *Plantago lanceolata* (Plantagenaceae). This shift occurred quite rapidly, going from 5 percent to 53 percent of females showing postalightment preference for the exotic plant in eight generations (Singer, Thomas, and Parmesan 1993). Early in the shift (1983–1984), postalightment oviposition preference measured in the field and laboratory showed a heritability of 0.90, based on mother-daughter regression, although this may be an overestimate if there were maternal effects (Singer, Ng, and Thomas 1988). Apparently larvae were preadapted to doing well on the exotic species, so no genetic changes were required in larval performance (Thomas et al. 1987). Indeed, larvae did much better on the exotic host than on *C. parviflora* because the exotic host matched butterfly phenology better (Singer 1984). By 1985, there was an interaction between oviposition preference and host plant that explained 32 percent of variation in larval performance (measured as weight gain), with larvae doing better on the host plant their mothers preferred for oviposition (Singer, Ng, and Thomas 1988). Better larval performance on the exotic and high heritability of oviposition preference, together with the weaker correlation between preference and performance, explain why this shift was so rapid (Singer et al. 2008). Another population of *E. editha* shifted from most females preferring to oviposit on one native plant, *Pedicularis semibarbata* (Scrophulariaceae), reduced in abundance by logging, to most females preferring to oviposit on a different native plant, *Collinsia torreyi* (Scrophulariaceae), increased in suitability by logging (Singer, Thomas, and Parmesan 1993). As with the shift to an exotic, the change was in postalightment preference and occurred rapidly, in twelve generations for this population (Singer and Thomas 1996). However, these preferences differed between patch types (rocky outcrops with *P. semibarbata* vs. logged areas with *C. torreyi*), with females ovipositing preferentially on the plant most abundant and suitable in their patch type (Singer and Thomas 1996). Interestingly, the oviposition frequencies switched back to the starting point when succession occurred in the logged patches and *C. torreyi* ceased being so suitable. Two conclusions about genetic architecture of host range in *E. editha* can be drawn from the rapidity of evolution in these populations. First, selection was strong, and second, there was either substantial genetic variation in the starting populations, or mutations readily supplied such variation; if the latter was the case, it suggests few genes with simple interactions were involved.

Other Systems

In laboratory experiments with full-sib families, larvae of *Depressaria patinacella* (Oecophoridae) showed genetic variation in survival on diets with fruits from their original host, *Pastinaca sativa* (Apiaceae), and those of a novel host, *Heracleum lanatum* (Apiaceae) (Berenbaum and Zangerl 1991), as well as in metabolism of parsnip furanocoumarins at various concentrations (Berenbaum and Zangerl 1992). However, larvae showed no genetic variation in feeding preference, indicating that adaptation to plant allomones was physiological rather than behavioral (Berenbaum and Zangerl 1991, 1992).

In laboratory experiments on rice and corn strains of *Spodoptera frugiperda* (Noctuidae), larvae of both strains performed best on rice, with the rice strain performing poorly on corn but the corn strain performing well on both hosts (Prowell, McMichael, and Silvain 2004). Analysis of genotype by environment interactions of full-sib families within strains showed variation that would promote host-associated divergence (Pashley 1988).

Larch and pine host races of *Zeiraphera diniana* (Tortricidae) mate assortatively (Emelianov et al. 2003). Genome-wide variation in hybridization between these host races suggests selection for host use in small regions of the *Z. diniana* genome, implying that a limited number of genes are involved in using alternative hosts (Emelianov, Marec, and Mallet 2004). The two host races differed in oviposition on larch versus pine but gave the same electroantennogram response to their odors; however, the plants differed in the numbers and concentrations of stimuli that elicited

responses (Syed, Guerin, and Baltensweiler 2003). Thus both host races could distinguish both plant species, but their decisions about what to do with this information differed.

Within cedar and cypress host races of *Mitoura* (Lycaenidae), oviposition preference was strongly correlated with larval performance (Forister 2004), but this correlation was lost in the F_1 progeny of reciprocal crosses between cedar and cypress races (Forister 2005). Survival of hybrid larvae on cypress was identical to the cypress race, but hybrid survival on cedar was 30 percent lower than the cedar race. Hybrid females preferred to oviposit on cedar, the same host that resulted in the reduced survival of hybrid larvae. Thus, oviposition preference for cedar was dominant, with hybrid preference indistinguishable from the cedar race, but larval performance on cedar was recessive, with hybrid performance indistinguishable from the cypress race.

Comparisons between host races may end up being comparisons between species. For example, the mugwort and maize host races of *Ostrinia nubilalis* are genetically isolated (Martel et al. 2003; Bethenod et al. 2005), mate assortatively (Malausa et al. 2005), and have different sex pheromones that attract essentially only males from the same host race (Pelozuelo et al. 2004). Recently these *O. nubilalis* host races have been determined to be different species (Frolov, Bourguet, and Ponsard 2007). Whether they are host races or cryptic species, studying the genetic basis of differences in host use will be useful; indeed, crosses between closely related species with different host ranges may prove to be the most useful approach to determining the genetic architecture of host range.

In laboratory experiments with F_1 hybrids and backcrosses of three closely related species of *Yponomeuta*, oviposition on *Euonymus europaeus* (Celastraceae), the normal host of *Y. cagnagellus*, was partially dominant to oviposition on *Prunus spinosa* (Rosaceae), a normal host of *Y. padellus*, and *Malus domestica* (Rosaceae), a normal host of *Y. malinellus* (Hora, Roessingh, and Menken 2005). Reciprocal crosses gave the same results, indicating that the genes involved were autosomal rather than sex-linked. In these experiments, both *Y. padellus* and *Y. malinellus* laid some eggs on their nonhost *E. europaeus*, perhaps retaining willingness to oviposit on this host because species of Celastraceae appear to be the ancestral hosts for *Yponomeuta* (Menken 1996; Hora, Roessingh, and Menken 2005).

Colias eurytheme and *C. philodice* (Pieridae) appear to be distinct species with diagnosable differences maintained by assortative mating. Nevertheless, they hybridize, which may account for a lack of differences in their adaptation to several novel, introduced host plant species (Porter and Levin 2007). However, differences in genetic correlations and heritabilities for fitness components among host plants for the two species suggest that the genetic architecture of host use may differ between them (Porter and Levin 2007).

Conclusions

The current knowledge of the genetic architecture of lepidopteran host ranges is limited mostly to heritability estimates, dominance relationships, and location of genes on autosomes versus sex chromosomes, although QTL mapping studies are in progress. Heritabilities for oviposition preference and larval performance can be high (e.g., 60–90 percent) but also can be zero. Dominance relationships run the gamut from additive to overdominant. Larval performance tends to be controlled by autosomal genes and oviposition preference by sex chromosome genes, but this trend is weak. Finally, evidence is accumulating that differences in host range between closely related species and host races appear to have a relatively simple architecture, involving few segregating factors (e.g., fewer than 10) that may interact epistatically.

INTEGRATION OF LARVAL AND ADULT TRAITS

Much attention has been devoted to the relationship between oviposition preference and larval performance because of its implications for host range evolution and speciation. With rare exceptions, lepidopteran adults suck nectar and sometimes eat pollen (if they feed at all), but their larvae chew

on plant tissue. Adults choose their own food by sight, smell, and taste (Ramaswamy 1988; Fitt 1991) and may feed on nectar from a variety of host plants unsuitable for larval development. Where females oviposit is determined in part by visual appearance, but primarily by the smell and taste of the surfaces of intact plants. Larval feeding decisions are largely determined by the odor and taste of intact plant surfaces and macerated tissues, though larvae may also use visual cues when moving from one plant to another. Whether larvae thrive on a host plant and produce fit adults depends on the interaction between their digestive systems, including ability to detoxify phytochemicals (Berenbaum and Zangerl 1992; Berenbaum, Cohen, and Schuler 1992; Hung et al. 1995; Rose et al. 1997; Stevens et al. 2000; Li et al. 2002; Wittstock et al. 2004; Zagrobelny et al. 2004; Berenbaum and Feeny 2008), and the plant tissues they ingest, as well as their nutritional requirements (Lee, Behmer, and Simpson 2006), especially for essential nutrients, or defensive chemicals they cannot produce themselves (Engler-Chaouat and Gilbert 2007). Host use involves a balance between two sets of traits: those of adults (e.g., location of hosts over a relatively large area, recognition and acceptance of suitable oviposition sites, and success in finding mates) and those of larvae (e.g., feeding on suitable hosts, recolonization of the host plant if dislodged, location of a new host plant if one is eaten up, and the ability to cope with plant defense compounds). Historically, it has been assumed that oviposition choice and larval performance are linked, so that females will tend to oviposit on plants that maximize larval performance, and oviposition preference will be influenced by larval host (e.g., Darwin 1909). However, given that these traits may be under different selection regimes and may be controlled by different sets of genes, complete integration of preference and performance may not be possible (Scheirs and De Bruyn 2002; Quental, Patten, and Pierce 2007).

The observed correspondence between oviposition preference and larval performance ranges from excellent to poor (for review, see Thompson and Pellmyr 1991). Recent work on *H. subflexa* has revealed that even extreme specialists may not always oviposit on the hosts that maximize larval fitness. In a common garden experiment involving seven *Physalis* species, oviposition preference of wild *H. subflexa* females did not correlate with larval performance (Benda 2007). *Physalis pubescens* was the species most preferred for oviposition, but larval feeding was greatest on *P. angulata* and *P. philadelphica*, which were less preferred for oviposition. On seventeen naturally occurring *Physalis* species in Mexico (the center of *Physalis* diversity), larval densities of *H. subflexa* on *P. pubescens*, *P. angulata*, and *P. philadelphica* were indistinguishable, and far greater than on the other ten *Physalis* species infested by *H. subflexa* larvae (Bateman 2006). In laboratory bioassays, *H. subflexa* larvae survived best on *P. angulata* (46 percent of neonates survived to pupation) but less well on both *P. pubescens* (34 percent) and *P. philadelphica* (30 percent). Interestingly, poor decision making was not restricted to adults: Larval feeding also failed to reflect performance reliably. In assays on thirteen *Physalis* species, larval mortality from starvation (with no attempt to feed) was quite high, ranging from 25 percent to 83 percent among plant species (Bateman 2006). If larvae had failed to feed only on plant species where performance was poor, one might conclude that refusal to feed on these suboptimal hosts was adaptive. In fact, the relationship between willingness to feed and survival to pupation was not consistent: Only 52 percent of neonates attempted to feed on *P. angulata*, but 89 percent survived to pupation; in contrast, 75 percent of neonates attempted to feed on *P. philadelphica*, but only 43 percent survived to pupation. It is unclear why larvae would refuse to eat suitable plants, especially in the absence of other choices. When presented with artificial diet, 95 percent of neonates fed, and their strikingly lower willingness to feed on plant material may reflect variation in larval sensitivity to species-specific plant compounds (Bateman 2006). In any case, it appears that neither oviposition preference nor larval feeding is fine tuned to larval performance in the specialist *H. subflexa*.

Given the difference in selection between larvae and adults, it is perhaps not surprising that preference and performance appear to be controlled by different genes (Thompson, Wehling, and Podolsky 1990; Sheck and Gould 1993, 1995, 1996). Even in cases with a strong correlation between preference and performance, this correlation appears to reflect independent selection on these traits, rather than a shared genetic basis. If larval and adult host use traits were controlled by genes on the

same chromosome, physical linkage might allow them to evolve in concert. However, genes affecting larval performance have consistently mapped to autosomes (Hagen 1990; Thompson, Wehling, and Podolsky 1990; Sheck and Gould 1996; Forister 2005), while genes affecting oviposition preference are less consistent, mapping sometimes to sex chromosomes and sometimes to autosomes (Sheck and Gould 1995; Forister 2005; Hora, Roessingh, and Menken 2005). Many traits associated with adult behavior (e.g., male response to pheromones: *Ostrinia* [Dopman et al. 2005]; female mate choice: *Colias* [Grula and Taylor 1980], Arctiidae [Iyengar, Reeve, and Eisner 2002]; female oviposition preference: *Papilio* [Thompson 1988; Scriber, Giebink, and Snider 1991], *Polygonia* [Nygren, Nylin, and Stefanescu 2006]) are sex linked, specifically to the male (Z) sex chromosome, suggesting that genes found on the Z chromosome may contribute disproportionately to the evolution of reproductive isolation and thus be important in speciation (Sperling 1994; Prowell 1998). However, sex linkage of oviposition preference may depend on the geographical scale of comparison: Janz (1998) found that variation between two populations of *Polygonia c-album* with different host specificity was sex linked, whereas Nylin et al. (2005) found strong variation in oviposition preference among females in a single population, but no evidence for sex linkage. Regardless of the autosomal versus sex chromosomal basis of oviposition preference, all research to date has suggested that oviposition preference and larval performance are controlled by genes on different chromosomes.

NEUROBIOLOGY OF HOST RANGE

Although some host-use genes (e.g., those involved in larval detoxification of plant allomones) may affect only one life stage, others probably act in both larvae and adults. Most notably, both larvae and adults use smell and taste to evaluate potential hosts, so genes involved in olfaction and gustation are likely to affect both egg laying and larval feeding. That the chemosensory systems of adults and larvae are often in harmony is demonstrated by females' generally ovipositing on plants where their larvae are willing to feed. In *Papilio*, adult oviposition and larval feeding are stimulated (or deterred) by the same chemicals, suggesting that the same chemosensory genes are responsible for host-use decisions in adults and larvae (Ono, Kuwahara, and Nishida 2004; Nishida 2005). Furthermore, P450s degrade odorants in adult *Papilio* as well as detoxify plant allomones in larvae (Ono, Ozaki, and Yoshikawa 2005) and may provide a link between adult oviposition and larval performance (Berenbaum and Feeny 2008).

Chemoreceptors are broadly classified as members of either the olfactory (Or) or gustatory (Gr) receptor subfamilies. Olfactory processing is mediated by olfactory binding proteins (OBPs) secreted into the aqueous lymph of sensilla and olfactory receptor proteins (ORPs) embedded in the membranes of olfactory receptor neurons (ORNs) that innervate sensilla. A thorough review of the neurobiology of insect olfaction is beyond the scope of this chapter (for recent reviews, see Mustaparta 2002; Chyb 2004; Rützler and Zwiebel 2005; Hallem, Dahanukar, and Carlson 2006; see Chapter 9 for a discussion of the phylogenetics of lepidopteran chemoreception genes). Briefly, odor molecules pass through the pores of olfactory sensilla on antennae and maxillary palps (the primary and secondary olfactory organs), are transported by an OBP to the membrane of an ORN, where they bind to an ORP, inducing an action potential that propagates along the axon of the ORN. While the dendrites of ORNs innervate the sensilla, their axons project into the glomeruli of the antennal lobe. Thus, stimulation at the periphery is quickly conveyed to the higher processing areas of the central nervous system.

ORPs are highly diverse, with many sharing less than 20 percent amino acid similarity. This diversity long delayed their discovery in insects, as homology-based similarity searches using known mammalian ORP sequences were unsuccessful. A combination of bioinformatic (Clyne et al. 1999) and genetic (Vosshall et al. 1999) approaches finally identified *Drosophila* ORPs, including sixty *Drosophila* Or genes. Identification of olfactory receptors in Lepidoptera has proved challenging because of low sequence similarity to Or genes in other organisms. Krieger et al. (2002) identified nine candidate ORPs by screening an *H. virescens* antennal cDNA library for proteins with partial

sequence similarity to *Drosophila* ORPs and used in situ hybridization to find which candidates were expressed in ORN. These newly identified proteins had very low amino acid sequence similarity with any identified Or genes, and homology was restricted to small regions of *Drosophila* ORPs. More promising for identification of adult chemoreception genes in Lepidoptera are the findings of Wanner et al. (2007) with *Bombyx mori*. Once *H. virescens Or* genes were identified, they used traditional measures of sequence similarity to identify forty-one candidate ORPs in the newly released *B. mori* genome, many of which appear orthologous with *H. virescens* ORPs (for details, see Chapter 9).

It is unclear what role OBPs and ORPs play in determining the host range of lepidopterans. In *Drosophila*, some ORPs are narrowly tuned to a single odor and some are more broadly tuned. Each ORP is expressed in a subset of three to fifty ORNs, and the response properties of ORNs arise from differences in the ORPs they express (de Bruyne and Warr 2006). Many ORNs respond to the same odor, and thus one odor typically activates multiple receptors (Hallem, Ho, and Carlson 2004; Goldman et al. 2005). This result helps explain why flies with an engineered deletion of an odor receptor often show normal olfactory-mediated behavior (Elmore et al. 2003). In *Drosophila* and probably Lepidoptera, specificity of response to odors relies on combinatorial discrimination of odorants, which has been observed in the first center of neuronal integration, the antennal lobe (Ng et al. 2002; Wang et al. 2003). Behavioral changes can be induced by the inactivation of selected subsets of olfactory neurons (Suh et al. 2004), and one recent study demonstrated that overexpression of a single odor receptor resulted in reduced behavioral avoidance of benzaldehyde, a compound involved in host avoidance and attraction in some Lepidoptera (Stortkuhl et al. 2005).

OBPs were first discovered in the antenna of the moth *Antheraea polyphemus* (Vogt and Riddiford 1981) and have since been identified in *H. virescens* (Krieger et al. 2002), *S. exigua* (Xiu and Dong 2007), *O. nubialis* (Coates, Hellmich, and Lewis 2005), *M. sexta* (Vogt et al. 2002), and other species. A variety of biochemical roles have been proposed for OBPs, including the transport of odorants through sensillum lymph to ORPs and the deactivation of odorants following receptor activation (Park et al. 2000); whether OBPs will prove to be involved in multiple processes in Lepidoptera remains to be seen. In *Drosophila*, recent work by Matsuo et al. (2007) suggests that OBPs may be involved in host range. They examined the genetic basis of oviposition choice in *D. sechellia*, a specialist on *Morinda citrifolia*, which is toxic to the closely related *D. melanogaster*. *Drosophila sechellia* is preferentially attracted to *M. citrifolia* fruit, while *D. melanogaster* is deterred by its odor. Using targeted gene knockout and replacement, they replaced two *D. melanogaster* OBP genes (*Obp57d* and *Obp57e*) with the *D. sechellia* versions. In the resulting transformed flies, oviposition preference closely mirrored that of *D. sechellia*. While such manipulations are not yet possible for any lepidopteran species, OBPs and ORPs are attractive candidate genes for explaining differences in host plant use.

In discussions of host plant acceptance, the role of larval choice is frequently overlooked. Although larvae are less mobile than adults, they may also be more motivated to find optimal hosts. As with adults, progress is being made in understanding the neurophysiology of host plant recognition and acceptance by larvae. Larval host choice appears to be based on a small set of gustatory receptors on antennae, maxillary palps, and epipharynx (Hanson and Dethier 1973; de Boer 1993, 2006; Glendinning, Valcic, and Timmermann 1998; Schoonhoven and van Loon 2002; Schoonhoven 2005). Gustatory sensilla, which are also found in adults, are innervated by four gustatory receptor neurons (GRNs), each responding only to sweet, salt, or water stimuli (Dethier 1976). The axons of GRNs project into the subesophogeal ganglion of the central nervous system, the first relay center of taste processing in the brain. Gustatory sensilla have been studied in a wide variety of insects, including moths and butterflies (Zacharuk 1980). To date, Gr genes have only been identified in two Lepidoptera: *H. virescens* (Krieger et al. 2002) and *B. mori* (for details, see Chapter 9). In *Drosophila*, sixty Gr genes have been identified (Robertson, Warr, and Carlson 2003), but receptor specificity has been determined for only a few of these (i.e., sugar receptors, Dahanukar et al. 2007; carbon dioxide receptors, Jones et al. 2007; Kwon et al. 2007; and bitter receptors, Moon et al. 2006).

The proteins encoded by identified Gr genes are, like OBPs and ORPs, extremely divergent in sequence, sharing as little as 8 percent amino acid identity (Scott et al. 2001). As with Or genes, Gr genes show much higher levels of sequence homology within Lepidoptera than between, for example, Lepidoptera and Diptera. Thus the increasing availability of Lepidoptera-specific resources (e.g., ButterflyBase, Papanicolaou et al. 2008; and the *B. mori* genome project, Mita et al. 2004; Xia et al. 2004) should make it easier to identify these genes in a wide variety of lepidopterans.

Given that host selection/acceptance is mediated by a balance of phagostimulatory and deterrent inputs (Schoonhoven 1987), a simple (i.e., single-gene) explanation of host range is unlikely. In many cases, the experience of an insect with its environment interacts with its genome to produce the observed host range. The larvae of *Pieris rapae* and *Manduca sexta* are polyphagous at hatching and become oligophagous only after exposure to a host-specific compound (a glucosinolate for *P. rapae* [Renwick and Lopez 1999] and indioside D for *M. sexta* [del Campo et al. 2001]). Presumably, following this exposure, plants lacking the relevant compound are deterrent (alternatively, only plants with the compound are stimulatory). In *M. sexta*, changes in the activity of the peripheral nervous system are known to occur after exposure to indioside D (del Campo and Miles 2003), but the mechanism by which these changes affect larval behavior is unknown.

The possibility that changes in host range are caused by changes in sensitivity to deterrent or stimulatory compounds is supported at the behavioral level (Bernays and Chapman 1987; Bernays et al. 2000). For example, the presence of benzaldehyde had no effect on feeding in *Y. cagnagellus*, a species that retains the original ancestral association with Celastraceae (which do not contain benzaldehyde), but stimulated feeding in species in a more derived clade that has shifted to the benzaldehyde-containing Rosaceae (Roessingh, Xu, and Menken 2007). Interestingly, such changes in peripheral sensitivity to host-associated chemicals appear to be a consequence rather than a cause of shifts in host range. In studies of both larvae and adults, species with widely divergent host ranges appear to have similar receptor neuron sensitivities. In larvae of *H. subflexa* and *H. virescens*, interspecific behavioral differences could not be attributed to differences in sensory neuron responses to stimulatory or deterrent compounds (Bernays and Chapman 2000; Bernays et al. 2000). In the generalists *H. armigera* and *H. virescens* and the specialist *H. assulta*, four types of ORNs in adult females of each species responded to the same four volatile plant chemicals, and each type of neuron, though narrowly tuned to a single molecule, showed some response to closely related molecules (Stranden et al. 2003). In both larvae and adults, species-specific host acceptance appears to depend on differences in central processing of sensory input, so changes in host range may depend upon changes in the central nervous system (Bernays and Chapman 1987; Bernays et al. 2000; Chyb 2004).

Although the mechanisms causing Lepidoptera with different host ranges to produce very different behaviors from the same peripheral input have not yet been identified, such work is under way in *Drosophila*. Melcher and Pankratz (2005) have identified a neuropeptide (coded by the *hugin* gene) expressed in gustatory interneurons that link peripheral receptor neurons with motor neurons in the ventral nerve cord and the pharyngeal apparatus. Output from these *hugin*-expressing interneurons appears to integrate taste, the endocrine system, higher-order brain centers, and motor output to modify feeding. As with the olfactory and gustatory genes, identification of lepidopteran neuropeptides may depend on the development and exploitation of Lepidoptera-specific resources. In the search for pheromone biosynthesis activating neuropeptide receptor genes, for example, very low levels of similarity were found between lepidopteran and *Drosophila* sequences, but similarity within the Lepidoptera was high (Zheng et al. 2007).

DIRECTIONALITY OF HOST RANGE EVOLUTION

Most Lepidoptera have relatively narrow host ranges, feeding on a small fraction of available plants. This preponderance of specialists may reflect host-associated fitness trade-offs (Jaenike 1990), selection by natural enemies (Bernays and Graham 1988), or neural constraints (Bernays

2001). Neural constraints (i.e., limitations of insect nervous systems that restrict the rate of information processing; Dukas 1998) might be the proximate means by which many checks on host range operate because insects with broad host ranges may be less efficient at correctly accepting or rejecting plants (and choose plants on which their fitness is reduced) or may simply take longer to choose hosts (thus increasing their exposure to natural enemies). The ability of adult females to select the best oviposition sites depends on accurately assessing host quality, and generalists seem to perform poorly compared to specialists. In assays of three specialist and two generalist nymphalid species on nettles of different quality, specialist females oviposited preferentially on high-quality nettles, but generalist females did not (Janz and Nylin 1997). All larvae performed poorly on low-quality nettles, so the poor choices made by generalist females reduced their reproductive success.

Larval performance also varies between specialists and generalists in a manner consistent with the neural constraints hypothesis. Bernays et al. (2000) found that larvae of the specialist *H. subflexa* rejected toxic diets untasted or after a single bite, but larvae of the generalist *H. virescens* rejected such diets only after extensive feeding. Apparently, the specialist relied on swift sensory evaluation of the diet, whereas the generalist relied on negative postingestive effects. This "eat now, decide later" approach of *H. virescens* larvae greatly increased their risk of consuming fatal doses of toxins. Inefficient decision making can also lead to reduced feeding opportunities: In assays of larval foraging behavior of two specialist and two generalist arctiid species, generalist larvae took much longer to accept or reject a plant and rejected many suitable host plants (Bernays, Chapman, and Singer 2004).

Although Mayr (1963) considered host range evolution to be unidirectional and irreversible, with generalists giving rise to specialists and specialization a dead end, more recent work has shown this to be false (Nosil and Mooers 2005). Instead, transitions from specialist to generalist and generalist to specialist occur freely and are not constrained by phylogeny (Winkler and Mitter 2008). For example, optimization of host-use traits on the phylogeny of Nymphalini suggests that ancestral specialization on Urticales was followed by frequent expansions and contractions of host range (Janz, Nyblom, and Nylin 2001). Furthermore, larvae of many species could feed on plants outside their current host range, with a strong bias toward plants used as hosts by other species of Nymphalini (Janz, Nyblom, and Nylin 2001). Such a bias is consistent with the striking conservatism in host use in most Lepidoptera. The observation that closely related insects often use closely related plants is well supported by phylogenetic reconstructions (e.g., Mitter and Farrell 1991; Winkler and Mitter 2008) and probably results from retention of ancestral host-use genes. Novelty, however, is also a common theme for host-use evolution. In the Nymphalini, some "extreme" host shifts to plant families outside the ancestral host range of either the Nymphalini or their close relatives in the *Nymphalis-Polygonia* clade occurred. A similar phenomenon is found with the Troidini tribe of Papilionidae, in which host range reflects neither host plant phylogeny nor plant secondary chemistry (Silva-Brandao and Solferini 2007). Instead, Troidini host range is strictly opportunistic, and increases in geographical range are strongly correlated with increases in the number of plant species used.

The evolution of host range probably reflects both the constraints of phylogeny and the constructive effects of natural selection, changing in response to the availability and adaptive value of particular host plants. Interestingly, shifts to novel host plants are associated with increased speciosity in *Polygonia*, where clades that shifted to novel host plants were more speciose than sister clades that used only hosts from the ancestral group (Weingartner, Wahlberg, and Nylin 2006). Such patterns are consistent with expansions in host plant range driving the elevated diversification rates observed in Lepidoptera and other phytophagous insects. In one recent analysis of 145 phytophage speciation events, fully half of the events were accompanied by shifts to new host plant species (Winkler and Mitter 2008).

FUTURE PROSPECTS

Our current understanding of the genetic architecture of host range is limited. Although differences in host range between closely related species and host races appear to have a relatively simple architecture, this conclusion awaits corroboration by more detailed analysis of the actual genes involved. Differences in sequence and expression of detoxification enzymes and chemoreceptor proteins have been implicated in differences in host range, but their full roles remain to be determined. Further advances in our understanding will require either much larger experiments or new approaches—and probably both. Crosses between closely related species or races that differ in host range provide the strong phenotypic differences and distinct molecular markers that together greatly aid in the identification of the genes responsible for differences in host range. Furthermore, it is exactly these differences in host range between recently diverged populations and species that are most intriguing. The most promising systems for this approach have involved species and populations in the genera *Euphydryas*, *Helicoverpa*, *Heliothis*, and *Papilio*.

Three main strategies show great promise for delineating in more detail the genetic architecture of differences in host range: (1) more and finer-scale genetic mapping of QTL, including combined genetic and physical mapping, (2) analysis of sequence and expression differences between closely related species or races that differ in host range, and (3) the effects of targeted disruption of candidate genes.

Genetic mapping of QTL is a powerful technique for determining the number and interaction of loci affecting quantitative traits (for review, see Lynch and Walsh 1998). However, QTL mapping might be better named QTR (quantitative trait region) mapping, because the number of genes between markers flanking QTL may be large. Many researchers have argued forcefully for the need to go beyond QTL mapping to identify the specific genetic changes underlying adaptive divergence (e.g., Remington, Ungerer, and Purugganan 2001; Orr 2005). Going from QTL to candidate gene can be quite challenging, especially when several different QTL affect phenotypes. Even if a single QTL is strongly implicated, sequencing the region between flanking markers in a nonmodel organism can prove difficult.

The development of whole-genome integrated physical/genetic maps (Chang et al. 2001; Yamamoto et al. 2006) can significantly accelerate map-based (positional) cloning of genes underlying QTL. Furthermore, sequencing has recently become much easier and cheaper with the introduction of ultrahigh-throughput technologies such as the Genome Sequencer FLX System (454-Life Sciences/Roche Applied Science, Indianapolis, Indiana, USA), Illumina Genome Analyzer (Illumina, San Diego, California, USA), and the SOLiD System (Applied Biosystems, Foster City, California, USA). Vera et al. (2008) used 454 pyrosequencing to generate approximately half a million high-quality reads from the genome of the Glanville fritillary, *Melitaea cinxia* (Nymphalidae). BLAST searches against the *B. mori* genome resulted in about nine thousand hits. If, as has been estimated for *B. mori*, most Lepidoptera have about eighteen thousand genes, then at least half of all genes in the *M. cinxia* genome have high levels of homology with *B. mori*. Vera et al. (2008) were able to detect a large number of sequence polymorphisms, a valuable source of genetic markers for QTL mapping and population genetic analysis. In addition, the availability of large-scale transcriptome information will allow species-specific microarrays to be constructed. Assembly of the short reads from these new technologies is a problem, but approaches like paired-end sequencing and the use of scaffolds from related species should ease assembly (Goldsmith, Shimada, and Abe 2005).

Improved techniques for fluorescence in situ hybridization (FISH) using genomic bacterial artificial chromosomes (BACs) as probes promise a renaissance in the cytogenetics of Lepidoptera, transforming their small, uniformly sized, undifferentiated chromosomes into powerful tools for the analysis of genome organization (Yoshido et al. 2005; Yasukochi et al. 2006; Sahara et al. 2007). Already, genetic mapping combined with BAC-FISH has revealed synteny in a variety of lepidopterans (Jiggins et al. 2005; Kaplan et al. 2006; Lee and Heckel 2007; Sahara et al. 2007). Integrated genetic/physical maps and this synteny may make it possible to use the well-mapped

and sequenced genome of *B. mori* to find candidate genes in chromosome segments delineated by common anchor loci.

For candidate genes, expression analysis combined with genetic mapping can determine whether the candidates map to the same region as QTL associated with phenotypic differences. The sequence differences among detoxification enzymes and olfactory receptor proteins can be quite large, so they should be readily distinguishable in expression analyses. An alternative method for testing gene function is to silence the expression of candidate genes using RNA interference (RNAi), which works by inducing intracellular enzymes that destroy native mRNA homologous to introduced double-stranded RNA (Bettencourt, Terenius, and Faye 2002). Several Lepidoptera species have been genetically transformed (Tamura et al. 2000; Thomas et al. 2002; Imamura et al. 2003; Marcus 2005), opening up the potential to develop RNAi constructs that express endogenously in the appropriate tissue or developmental stage.

The new and developing techniques in molecular genetics have the potential to move lepidopteran genetics beyond the limited world of model organisms and into one that better reflects the great diversity of lepidopteran biology. Unlike earlier insect model systems, existing research on Lepidoptera is deeply rooted in attempts to understand the evolution of traits that allow insects to adapt to a variety of environments. The combination of the vast knowledge of the behavior, ecology, and phylogeny of many species of Lepidoptera that has accumulated over the last half century with the rapidly expanding availability of cutting-edge genetic and genomic technologies promises exciting progress in our understanding of the genetic architecture of lepidopteran host ranges in the near future.

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